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Hepato-protective Efficiency of Ethanol Leaf Extract of *Moringa* oleifera Lam. against Hydrocarbon Exposure

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Hydrocarbon exposure has been reported to have deleterious effect on human health including damaging the organs. This study sought to investigate the hepatoprotective efficiency of ethanol leaf extract of *Moringa oleifera* against hydrocarbon exposure. Twenty adult Wistar rats were used for this study. They were divided into four groups and treated accordingly: animals in group A were fed with 100 g of standard feed only (Control group), Group B with 100 g of standard feed + 4 mL of crude oil, group C with 100 g of standard feed +10 g of extract of *M. oleifera* and group D with 100 g of standard feed + 4 mL of crude oil + 10 g of extract of *M. oleifera* leaves. At the end of thirty days treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture. Biochemical parameters were determined using standard methods. Crude oil significantly increased hepatic biomarkers as well as oxidative stress biomarker of animals used in this study when compared with the control group at p<0.05 but this effect was neutralized by *M. oleifera* leaf extract. This indicates that crude oil damaged the liver and increased the generation of free radicals. This might be what happens in humans and animals living in the Niger Delta area of Nigeria who are continuously exposed to crude oil following exploration. *M. oleifera* was able to alleviate this effect, thus making it a potent hepatoprotective agent against hydrocarbon exposure.

Keywords: *Moringa oleifera* Lam., hydrocarbon exposure, Liver Damage, Hepatoprotective Agent.

INTRODUCTION

In the Niger Delta area of Nigeria some rural dwellers are exposed to crude oil because they use the chemical in various forms to treat a variety of ailments. They do this either by ingesting the crude oil or taken in combination with other substances (Dienye et al., 2012). In addition, humans get exposed to crude oil by consuming contaminated food, either directly or through the food chain

(Sunmonu and Oloyede, 2007). Crude oil is injurious to animal's health (Achuba and Ogwumu, 2014a), which can be acute or chronic. Acute exposure of animals to crude oil usually result in eye irritation, nausea, vomiting, diarrhea and confusion, while chronic effects of petroleum hydrocarbon include decreased immune function, organ damage, biochemical and physiological abnormalities (Unwin et al., 2006; Tormoehlen et al., 2014). In fact, petroleum hydrocarbon causes metabolic imbalances in experimental animals (Achuba et al., 2016). In addition, crude oil had been implicated in the alteration of haematological parameters in animal models (Ita and Udofia, 2011). Moreover, researchers have shown that antioxidants such as vitamins (Achuba and Otuya, 2006), palm oil (Achuba and Ogwumu, 2014b), and honey (Achuba and Nwokogba, 2015) can be used to attenuate petroleum hydrocarbon toxicity in animals.

Liver is the major organ which plays key roles in processing critical biochemical and physiological phenomena including metabolism and detoxification of endogenous and exogenous compounds, such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply (Mahmood et al., 2014). Hepatic injury could occur by hepatotoxic agents including drugs, alcohol, hydrocarbon and viral infections (Saleem et al., 2018). Liver diseases like jaundice, cirrhosis and fatty liver have been public health concern across the world. Prevalence of chronic liver disease worldwide is 18.5% and cirrhosis is 4.5 to 9.5% while 2 million people die each year. In terms of medication, conventional or synthetic drugs are limited. Moreover, they can have serious side effects (Rao and Rao 2006). Due to this fact, a huge number of medicinal plants have been used to figure out regenerative and hepatoprotective activity (Saleem et al., 2018). Approximately 160 phytochemical constituents originated from 101 plants have been reported to be potentially hepatoprotective (Chatterjee, 2000). At present, medicinal herbs have been a vital source of treatment of liver diseases such as hepatitis, cirrhosis, and loss of appetite (Devaraj et al.,

Moringa oleifera Lam. is the most widely cultivated species of the mono-generic family Moringaceae, which includes 13 species of trees and shrubs distributed in sub Himalayan ranges of India, Sri Lanka, North-eastern and South-western Africa,

Madagascar and Arabia. Moringa is also native to parts of West Africa particularly Nigeria (Monica et al., 2010). The whole Moringa oleifera plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation (Patel et al., 2010). The Moringa tree is a multifunctional plant. It has been cultivated in tropical regions all over the world for the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; high oil content (42%) of the seed which is edible, and with medicinal uses; the coagulant of seeds could be used for wastewater treatment (Monica et al., 2010).

Different parts of the *M. oleifera* (Mo) tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids (Amaglo et al., 2010), carotenoids (Saini et al., tocopherols (Saini et al., 2014a), 2014b), polyunsaturated fatty acids (PUFAs) (Saini et al., 2014c), highly bioavailable minerals (Saini et al., 2014d), and folate (Saini et al., 2016). Among glucosinolates, 4-O-(a-L-rhamnopyranosyloxy)benzylglucosinolate (glucomoringin) is the most predominant in the stem, leaves, flowers, pods and seeds of *M. oleifera* (Amaglo et al., 2010). Although in the roots, benzyl glucosinolate (glucotropaeolin) is the most prominent. The highest content of glucosinolate is found in the leaves and seeds. The enzymatic catabolism of glucosinolates by the endogenous plant enzyme myrosinase produces isothiocyanates, nitriles, and thiocarbamates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects (Anwar et al., 2007). In the leaves, the amount of quercetin and kaempferol was found to be in the range of 0.07-1.26 and 0.05-0.67 %, respectively. The potent antioxidant activity of Moringa is attributed to the high concentration of these polyphenols. Medicinally, the antioxidant, wound healing, hypotensive, and diuretic effects of this plant have been reported (Guevara et al., 1999). Airaodion et al., (2019a) have reported its protective effect on haematological indices in crude oil treated diet (Figure 1). Previous studies have reported the antioxidant (Limon-Pacheco and Gonsebatt, 2009), anti-inflammatory and pharmacological properties of M. oleifera (Amaglo et al., 2010). Furthermore, Awodele et al., (2012) worked on the toxicological evaluation of the aqueous extract of Moringa



Figure 1. *Moringa oleifera* Leaves (Airaodion et al., 2019a).

oleifera Lam (Moringaceae). Oyedepo et al., (2017) evaluated the anti-hyperlipidemic effect of aqueous leaves extract of *M. oleifera*, while Choudhary et al., (2013) assessed the antiulcer potential of *M. oleifera* root bark extract in rats. *M. oleifera* leaf has been reported to be potent in the prevention of peptic ulcer (Airaodion et al., 2019b). Furthermore, Airaodion et al., (2019c) reported that the combination of *M. oleifera* leaves and turmeric root is more potent in the prevention of peptic ulcer. This study therefore sought to evaluate the hepatoprotective efficiency of ethanol leaf extract of *Moringa oleifera* against hydrocarbon exposure in Wistar rats.

MATERIALS AND METHODS

Plant Material and Leaf Extract Obtention

Fresh plants of *M. oleifera* were obtained from Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants and air dried at room temperature in an open laboratory space for ten days and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the methods described by Airaodion et al., (2019d) with a yield of 2.55 g which represents a percentage yield of 10.20%. The extract was preserved in the refrigerator for further analysis.

TREATMENTS AND EXPERIMENTAL DESIGN

Twenty adults male Wistar rats with body weight between 200 and 220 g were purchased from a private animal house in Osogbo, Nigeria. They were acclimatized for seven (7) days during which they were fed ad libitum with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiments. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Four treatments were evaluated in a completely random design with five replications, experimental unit was one rat in an individual cage. treatments were as follows:

Group A: 100 g of standard feed only (Control group)

Group B: 100 g of standard feed + 4 mL of

crude oil.

Group C: 100 g of standard feed +10 g of extract of *M. oleifera*

Group D: 100 g of standard feed + 4 mL of crude oil + 10 g of leaf extract of *M. oleifera*

At the end of thirty days treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determination of the hepatic marker enzymes activities and oxidative stress biomarker.

Determination of Hepatic Marker Enzymes Activities

Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Lactate Dehydrogenase (LDH) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel (1957).

Determination of Oxidative Stress Biomarkers

Determination of hepatic Lipid Peroxidation (LPO), Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) were carried out according to the methods previously described by Airaodion et al., (2019e).

STATISTICAL ANALYSIS

Results are expressed as mean \pm standard error of the mean (S.E.M.). The levels of homogeneity among treatments were assessed using One-way analysis of variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

RESULTS

The results of this study are presented in Tables 1 and 2.

DISCUSSION

Evaluation of liver function is very important when analyzing toxicity of drugs and plant extracts because of its relevance for the survival of the organism (Owoade et al., 2018). High levels of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) has been reported to be an indicator of hepatotoxicity or liver damage/diseases (Brautbar and Williams, 2002). Studies on the alterations of these enzymes might reflect the metabolic abnormalities and cellular injuries in some organs. The liver and kidney have extremely important function in detoxification and excretion of metabolic wastes and xenobiotics (Kaneko et al., 1999). Exposure to toxic chemicals causes alterations in some tissue enzyme activities (Gholipour-Kanani et al., 2013; Al-Ghanim, 2014). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are distributed extensively in several different organs and have important roles in carbohydrate and amino acid metabolic pathways and their activities is established to change under several physiological and pathological circumstances (Al-Ghanim, 2014).

In this study, the activities of AST, ALT, ALP and LDH were not significantly different when animals treated with *M. oleifera* extract only were compared with those of the control group at p<0.05. This might be due to the non-toxic nature of the plant. A

significant increase was however observed when the activities of AST, ALT, ALP and LDH in animals treated with hydrocarbon only were compared with those of the control and *M. oleifera* extract only groups at p<0.05 (Table 1). This might be an indication that hydrocarbon causes liver damage to the animals. This result is in agreement with the findings of Ogbuagu et al., (2019) who studied the prophylactic propensity of methanolic extract of Vernonia amvadalina leaves against acute ethanolinduced oxidative stress in Wistar rats. Furthermore. when animals fed crude oil and M. oleifera leaf extract were compared with those fed crude oil only, a significant decrease was observed in the activities of AST, ALT, ALP and LDH. This could be that M. oleifera extract has the potential to increase transcription of some genes involved in glucose uptake, glycolysis and lipogenesis (Towle et al., 1997). Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic adenosine monophosphate (cAMP) a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the Lac operon. Cyclic AMP (cAMP) is required to activate an allosteric protein called catabolite activator protein (CAP) which binds to the promoter CAP site and stimulates the binding of ribonucleic acid (RNA) polymerase to the promoter for the initiation of transcription, but cAMP must be available to bind to CAP which binds deoxyribonucleic acid (DNA) to facilitate transcription. In the presence of glucose, adenylase cyclase (AC) activity is blocked. AC is required to synthesize cAMP from adenosine triphosphate (ATP) (Todar, 2008). Therefore, if cAMP levels are low, CAP is inactive and transcription does not occur. Thus, the effect of glucose in suppressing these inducible enzymes is by lowering cyclic AMP level. The M. oleifera extract might have lowered cAMP in animals thus causing inhibition of these inducible enzymes. ALT is considered most reliable hepatocellular injury because it is solely confined to the liver, unlike AST and LDH which are also abundantly present in other body organs such as the kidneys, brain, and hearts (Johnson, 1995). The significant decrease observed in the activities of hepatic enzymes in crude oil and M. oleifera-treated animals when compared to those treated with crude oil only showed that *M. oleifera* protected the liver from damage by crude oil exposure. Report by Torres-Castillo et al., (2013) showed that M. oleifera is rich in phytochemicals and antioxidants. Its

Table 1. Effect of M.	oleifera	Leaves o	n Hepatic	Marker	Enzymes	of Animals	Exposed to	Hydrocarbon
for Thirty Days.					-		-	

Hepatic Marker Enzymes	Control	4 mL of Crude Oil only	M. oleifera Extract only	4 mL of Crude Oil + M. oleifera Extract
AST (IU/L)	122.92±11.11ª	153.27±14.32b	119.02±12.02a	124.91±6.02a
ALT (IU/L)	48.01±1.34ac	62.03±3.83 ^b	49.52±2.29a	51.24±2.19 ^a
ALP (IU/L)	18.00±0.83ac	27.03±1.35 ^b	17.87±2.01a	21.56±3.42°
LDH (IU/L)	179.21±13.05 ^a	211.10±12.84 ^b	188.01±10.22c	189.91±13.91°

Values are presented as Mean \pm S.E.M., where n = 5. Values with different superscript along the same row are significantly different at p<0.05.

Legend: AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, ALP = Alkaline Phosphatase, LDH = Lactate Dehydrogenase

Table 2. Effect of *M. oleifera* Leaves on Oxidative Stress Biomarkers of Animals Exposed to Hydrocarbon for Thirty Days.

Oxidative Stress Biomarkers	Control	4 mL of Crude Oil only	M. oleifera Extract only	4 mL of Crude Oil + <i>M. oleifera</i> Extract
LPO (nmol MDA/mg protein)	13.05±1.35 ^a	19.44±2.03 ^b	13.38±1.29 ^a	14.25 <u>+</u> 3.74 ^a
GSH (mg/mL)	6.51±0.22a	3.94 ±0.27 ^b	6.11±0.12a	5.89±1.94 ^a
CAT (Mm H ₂ O ₂ /mg protein)	12.63 <u>+</u> 1.94ª	23.84±2.47 ^b	14.00±2.83 ^a	13.88±3.22ª
SOD (U/mg protein)	10.28±1.21a	17.23±2.94b	9.86±1.14a	11.36±1.38a
GPX (U/mg protein)	9.22±1.09 ^{ac}	12.45 ±1.17 ^b	5.53±0.83°	10.96±2.53ab

Values are presented as Mean \pm S.E.M., where n = 5. Values with different superscript along the same row are significantly different at p<0.05.

Legend: LPO = Lipid Peroxidation, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, GPX = Glutathione Peroxidase

hepatoprotective efficiency against hydrocarbon exposure might be due to the presence of these phytochemicals and antioxidants.

Alkaline phosphatase (ALP) is involved in the hydrolysis of a wide range of phosphomonoester substrates. It is a marker enzyme for the plasma membrane and endoplasmic reticulum of tissues (Swarna and Ravindhran, 2013). It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculli, located in the plasma membrane. Since ALP hydrolyzes phosphate monoesters, its significant increase in ethanolinduced animals without pretreatment could

constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver (Akanji et al., 1993). Consequently, this may adversely affect the facilitation of the transfer of metabolites across the cell membrane of animals treated with crude oil only. However, this effect was rectified by treatment with *M. oleifera* leaf extract. The elevation in the activities of biomarkers such as ALT, AST and LDH in the liver tissue of animals treated with crude oil only might be due to cellular necrosis of hepatocytes, which causes increase in the permeability of the cell. Lactate dehydrogenase

(LDH) is an index of cell damage including hepatotoxicity and the endothelial disruption in blood vessel. The significant increase observed in the activity of LDH due to hydrocarbon exposure might be suggestive of the beginning of cytolysis, which is a possible indication of membrane damage including the endothelial membranes of blood vessels. This disruption of endothelial membrane, directly or indirectly includes the generation of reactive oxygen species in endothelial cells (Oyenihi et al., 2016). Free radicals attack unsaturated fatty acids in the membranes resulting in membrane lipid peroxidation which decreases membrane fluidity. leakage of enzyme and loss of receptor activity as well as damage membrane proteins leading to cell inactivation (Airaodion et al., 2019 e, f). As lipid peroxidation progressively increase, antioxidant defense system decreases equivalently resulting in oxidative stress. This suggests that the exposure to hydrocarbon might have weakened the liver membrane of the rats with subsequent penetration and elevation of the hepatic biomarker enzymes.

Hydrocarbon exposure may result in oxidative and nitrosative stress via elevation of NADH/NAD+ redox ratios, induction of nitric oxide synthase (NOS) and NADPH/xanthine oxidase (Airaodion et al., 2019 e. f). Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a common feature of both acute and chronic alcohol consumption (Ramezani et al., 2012). The presence of a high concentration of oxidizable fatty acids and iron in liver significantly contributes to ROS production. A rise in lipid peroxidation level is only identified if there is oxidative damage due to the increase in free radical generation. Generally, under normal conditions, the animals tend to maintain a balance between generation and neutralization of ROS in the tissues. But, when the organisms are subjected to xenobiotic stress, the rate of production of ROS including O₂, H₂O₂, OH⁻, ROO⁻⁻, exceeds their scavenging capacities. All the organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic Enzymatic antioxidant components. pathway consists of SOD, CAT and GPX. Superoxide anion $O_{\overline{z}}$ is dismutated by SOD to H_2O_2 , which is reduced to water and molecular oxygen by CAT or is neutralized by GPX, which catalyzes the reduction of H₂O₂ to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. Glutathione reductase (GR) regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of ROS as well as a substrate for other enzymes. Glutathione S-transferase (GST) conjugates xenobiotics with GSH for exclusion.

In this study, hydrocarbon exposure significantly elevated the malondialdehyde (MDA) levels in the liver indicating enhanced peroxidation breakdown of the antioxidant defense mechanisms. Decomposition products of lipid hydroperoxide such as malanoldehyde and 4-hydroxynonenal can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis. In this investigation, hepatic lipid peroxidation (LPO) activities show significant increase due to hydrocarbon exposure. Furthermore, extensive damage to tissues in a free radical mediated LPO results in membrane damage and subsequently decreases the membrane fluid content. Treatment with M. oleifera leaves reversed these alterations causing a significant decrease in MDA levels, suggesting its protective effects against hydrocarbon exposure. This is consistent with the study of Oyenihi et al., (2016) who reported the hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. It is also in agreement with the report of Airaodion et al. (2019e) who studied hepatoprotective effect of Parkia biglobosa on acute ethanol-induced oxidative stress in Wistar rats. This means that the mechanism of action of crude oil in the liver of animals is similar to that of ethanol. Glutathione (GSH) is tripeptide (L-αglutamylcysteinol glycine) which is highly abundant in all cell compartments and it is the major soluble antioxidant. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism (Livingstone and Davis, 2007). Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation. In this study, the significant decrease observed in the reduced glutathione level in animals treated with crude oil only directs the conjugation of GSH with acetaldehyde. This result corresponds with the findings of Airaodion et al. (2019 f) who recorded a significant decrease in the concentration of GSH following ethanol administration in Wistar rats. The significant increase in the glutathione levels in the liver of crude oil and M. oleifera-treated rats compared with animals treated with crude oil only may be due to the direct ROS—scavenging effect of M. oleifera or an increase in GSH synthesis. The

phytochemical content and antioxidant potential of *M. oleifera* reported by Torres-Castillo et al., (2013) might also be responsible in this activity.

Catalase (CAT) is another antioxidant which helps in the scavenging of free radical from the system. In this study, a significant increase was observed in the activity of catalase in animals exposed to crude oil only when compared with the control group. This might be that hydrocarbon exposure generated elevated ROS in the liver which CAT tend to combat, thereby increasing its activity. *M. oleifera* was able to reduce the ROS generation with subsequent decrease in CAT activity due to its high phytochemical content and antioxidant potential as reported by Torres-Castillo et al., (2013).

Superoxide dismutase (SOD) plays an important role in reducing the effect of free radicals attack, and SOD is the only enzymatic system quenching O_2 - to oxygen and H_2O_2 and plays a significant role against oxidative stress (Oh et al., 1998). These radicals have been reported to be deleterious to polyunsaturated fatty acids and proteins (Murray et al., 2003). In this study, no significant difference was observed in the activity of SOD in control animals and those treated with M. oleifera extract only when compared with those treated with crude oil + M. oleifera. This is suggestive that M. oleifera leaves is non-toxic and did not generate free radicals.

However, the activity of SOD in animals treated with crude oil only was significantly elevated when compared with those in control group and animals treated with crude oil + *M. oleifera* leaf extract. This might be that crude oil exposure generated ROS in the liver which SOD tend to combat thereby increasing its activity. *M. oleifera* was able to reduce the ROS generation with subsequent decrease in SOD activity due to its high phytochemical content as reported by Torres-Castillo et al., (2013).

Glutathione peroxidase (GPX) enzymatic antioxidant that acts as a defense mechanism against oxidative stress. In this study, no significant difference was observed in the activity of GPX in control animals when compared with those treated with crude oil combined with $M_{\rm c}$ oleifera leaf extract at p<0.05. However, the activity of GPX in animals treated with M. oleifera only was significantly reduced when compared with those treated with M. oleifera only. Contrarily, a significant increase was observed in the activity of GPX in animals treated with crude oil only when compared with control group. This might be that crude oil exposure generated ROS in the liver which GPX

tend to combat thereby increasing its activity. *M. oleifera* was able to reduce the ROS generation with subsequent decrease in GPX activity due to its high phytochemical content antioxidant potential reported Torres-Castillo et al., (2013).

CONCLUSION

The present study showed that long term exposure to hydrocarbon has the propensity to cause liver damage. In the same vein, ethanol leaf extract of *M. oleifera* has the potential to protect the liver against damage by hydrocarbon exposure. Thus, inhabitants of Niger Delta Area of Nigeria and indeed oil producing communities should consume *M. oleifera* leaves to protect their liver against damage.

Conflict of Interests

No conflict of interest exists in this research and publication.

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